Review Paper Examen critique

Dopamine genes and attention-deficit hyperactivity disorder: a review

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Objective: To review the results of genetic studies investigating dopamine-related genes in attention-deficit hyperactivity disorder (ADHD). **Data sources:** Papers (association/linkage, meta-analyses and animal model studies) were identified through searches of the PubMed database and systematically reviewed. **Data synthesis:** Consistent results from molecular genetic studies are pointing strongly to the possible link between 2 specific genes, the dopamine transporter (*SLC3A6*) and the dopamine receptor 4 (*DRD4*), and ADHD. **Conclusions:** The implication of *SLC6A3* and *DRD4* genes in ADHD appears to be one of the most replicated in psychiatric genetics and strongly suggests the involvement of the brain dopamine systems in the pathogenesis of ADHD. However, more work is required to further these findings by genotype-to-phenotype correlations and identify the functional allelic variants/mutations that are responsible for these associations. The role of other dopamine genes, which may have smaller effects than *SLC6A3* and *DRD4*, needs also to be determined.

Objectif: Examiner les résultats d'études en génétique portant sur les gènes liés à la dopamine chez les personnes atteintes du trouble d'hyperactivité avec déficit de l'attention (THADA). **Sources des données**: Des documents (études d'association ou de lien, méta-analyses et études sur modèle animal) recensés grâce aux recherches effectuées dans la base de données PubMed ont fait l'objet d'un examen systématique. **Synthèse des données**: Des résultats homogènes provenant d'études en génétique moléculaire indiquent nettement la possibilité d'un lien entre le THADA et deux gènes précis, soit le transporteur de la dopamine (*SLC3A6*) et le récepteur D4 de la dopamine (*DRD4*). **Conclusions**: Il semble que le lien entre les gènes *SLC6A3* et *DRD4* et le THADA soit l'un des plus souvent établis dans le domaine de la génétique en psychiatrie. Cela porte fort à penser que les systèmes de dopamine du cerveau interviennent dans la pathogenèse du THADA. Il faudra toutefois effectuer d'autres travaux afin de préciser ces constatations selon les corrélations génotype-phénotype et de cerner les mutations ou allèles fonctionnels qui causent ces associations. Il faut aussi déterminer le rôle d'autres gènes de la dopamine, dont les effets pourraient être moins importants que ceux des gènes *SLC6A3* et *DRD4*.

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Medical subject headings: alleles; attention deficit disorder with hyperactivity; case-control studies; dopamine; dopamine beta-hydroxylase; genes; genetic predisposition to disease; meta-analysis; minisatellite repeats; molecular biology; polymorphism, genetics; receptors, dopamine.

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Introduction

Attention-deficit hyperactivity disorder (ADHD) is a childhood onset, clinically heterogeneous disorder characterized by excessive motor activity, impulsiveness and inattention. Roughly 5%–10% of all school-aged children worldwide have ADHD,¹⁻⁴ and it is not uncommon for the condition to persist into adulthood.⁵ Although the etiology of ADHD is unknown, family, twin and adoption studies have demonstrated high familiality⁶⁻⁸ due mainly to shared gene effects.⁹ It is widely accepted that several genes, each contributing a small fraction of the total genetic variance, are implicated in ADHD.^{10,11}

Several lines of evidence indicate dopamine system dysfunction in the pathogenesis of ADHD. First, methylphenidate, amphetamine and other psychostimulant drugs that inhibit the activity of the dopamine transporter and increase synaptic levels of dopamine effectively control ADHD symptoms. Second, magnetic resonance imaging and single-photon emission computerized tomography studies¹² demonstrate abnormalities in neuroanatomical areas with rich dopamine innervations in ADHD children. Third, animal studies strongly suggest that abnormalities of dopamine neurotransmission may be pivotal in motor control^{13–16} and other neuropsychological¹⁷ functions purportedly affected in ADHD.

Recently, polymorphic sites at dopamine-related genes — encoding for enzymes, receptors and transporters, many of which cause observed alterations in protein function or structure — have been identified, prompting researchers to test their role in increasing the risk for ADHD. The main objective of this paper is to review the results of studies investigating dopamine-related genes in ADHD. We will review data relating each gene to ADHD or its major symptoms and summarize the literature specifically devoted to investigating the risk conferred by various alleles of the gene to the development of ADHD.

Methods

The search for susceptibility genes of small effect in a polygenic disorder such as ADHD has been approached in a number of ways. In contrast to many other psychiatric disorders, there were very few linkage studies in ADHD. Indeed, the only genome-wide scan for susceptibility loci among ADHD-affected sibling pairs was

published recently by the group of Smalley.¹¹ In this study, loci conferring a substantial amount of risk to develop ADHD in siblings of affected individuals (relative risk ≥ 2.5 as compared with the risk in the general population) were undetectable in 92% of the human genome, curtailing the possibility of a major susceptibility gene in ADHD. Only one other study investigating markers in the 20p11-p12 locus, syntenic to the mice 2q locus deleted in the coloboma mice model of ADHD, was published. No linkage was identified between ADHD and markers in this locus.¹⁸ Comings' extensive review of the molecular genetics of ADHD in 2001¹⁹ showed further evidence of polygenicity and limited variance explained by each gene implicated in the disorder.

Case-control association studies comparing frequencies of marker alleles in ADHD patients to those in unrelated control subjects are numerous. Data based on this type of analysis, however, are often difficult to interpret because of the possibility of population stratification, namely that ethnic differences in allele frequencies can contribute to observed differences between affected subjects and controls. Family-based association designs, in which parental or full sibling genotypes are used as "internal controls,"20 are favoured because they control for outside sources of variance, including ethnic variance in allele frequencies. Several statistics have been proposed to test for association between an allele in a candidate gene and a disease, including the haplotype-based haplotype relative risk (HHRR) test, in which alleles transmitted to affected children are compared with alleles that are not transmitted.21 Another test, the transmission disequilibrium test (TDT), is currently the most robust test for "linkage" with association and is designed to control for population subdivision and admixture,22 although the TDT may be statistically less powerful²³ and may result in some selection bias²⁴ compared with the populationbased case-control association design.

Papers included in this review were identified by searching journal abstracts available online through PubMed at the National Library of Medicine using a number of search keywords for each of the candidate genes, including: "association studies," "meta-analyses," "animal model" and the specific name of the gene (e.g., "DRD3" or "dopamine receptor 3"). Relevant papers that were not listed in the PubMed database but that we identified while reviewing papers listed in PubMed were also reviewed. We limited our search for papers published to English-language papers.

Results

Dopamine transporter gene (SLC6A3)

The dopamine transporter gene (*SLC6A3*) is of great interest given that methylphenidate is theorized to inhibit the function of this transporter by preventing presynaptic reuptake of dopamine. Giros et al¹³ developed a dopamine transporter knock-out (*Slc6a3*-KO) mouse, which displayed behavioural traits highly reminiscent of ADHD characteristics observed in humans. Indeed, *Slc6a3*-KO mice were spontaneously hyperactive, had higher levels of motor activity induced by stress compared with wild-type animals and were significantly calmed by the administration of amphetamine or methylphenidate. In addition, dopamine was found to remain 100 times longer in the extracellular medium of homozygous *Slc6a3*-KO mice than in heterozygous and wild-type animals.

The human (*SLC6A3*) gene was localized by Giros et al²⁵ and Vandenbergh et al²⁶ to chromosome 5p15.3. Sequence analysis of this gene revealed a VNTR (variable number of tandem repeats) polymorphism with a 40-bp unit repeat length, ranging from 3 to 11 copies.

Published association and linkage studies of the *SLC6A3* gene in ADHD humans are indicated in Table 1; all focused primarily on the 3' VNTR marker, in particular the 10-repeat (480-bp) putative high-risk allele, as well as the 9-repeat 440-bp allele. All of the studies investigated an association between *SLC6A3* and ADHD using either the TDT or the HHRR test. Interestingly, only 1 of 6 groups using the TDT identified linkage compared with 3 of 4 groups who applied

HHRR analysis. Although the underlying reason for such a discrepancy is largely unknown, linkage in the TDT studies may be difficult to detect if sample sizes are insufficient for each group. In addition to studies included in Table 1, Todd et al³⁵ examined association using the TDT in a population sample of twins. They found no significant disequilibrium of the VNTR alleles using a number of ADHD diagnostic systems.

Curran et al²⁹ recently combined available data from published studies of the VNTR polymorphism and, using the TDT, found evidence for association and linkage (odds ratio = 1.15, p = 0.06). Similarly, Swanson et al³⁶ combined the SLC6A3 data from 3 earlier studies to measure allele proportions of the 10-repeat VNTR polymorphism among ADHD populations. Using the HHRR method, a significantly greater frequency of the 10-repeat allele was observed compared with control groups, indicating that the transporter gene is likely to be implicated in the etiology of ADHD. Notwithstanding, other studies have suggested a limited association between the VNTR polymorphism and SLC6A3 expression in humans.³⁷ More studies with larger samples will be needed to further elucidate the role of SLC6A3 in ADHD.

Taking an interesting pharmacogenetic approach, Winsberg and Comings³⁸ have also reported that homozygosity for the 10-repeat allele of the *SLC6A3* was significantly increased in African-American children with ADHD symptoms who respond poorly to methylphenidate. Although promising, the results of this study should be considered cautiously because of several limitations discussed by the authors, including the fact that the assessment of therapeutic response to methylphenidate was based on an open trial.

Table I: Studies of the association between attention-deficit hyperactivity disorder (ADHD) and the SLC6A3 480-bp VNTR allele

Study	Location	No. of probands	Diagnostic system	Test of association	Linkage	Statistic	p value
Barr et al ²⁷	Canada	102	DSM-IV	TDT	_	$\chi^2 = 2.6$	0.06
Roman et al ²⁸	Brazil	81	DSM-IV	HHRR	_	$\chi^2 = 0.02$	0.88
Curran et al ²⁹	Turkey	111	DSM-IV	TDT	_	$\chi^2 = 0.93$	0.34
Curran et al ²⁹	United Kingdom	66	DSM-IV	TDT	+	$\chi^2 = 8.97$	0.001
Holmes et al ³⁰	United Kingdom	137	ICD-10, DSM-IV and DSM-III-R	TDT	_	OR = 0.89	0.59
Palmer et al ³¹	United States	209	DSM-IV and DSM-III-R	TDT	_	OR = 0.88	0.40
Daly et al ³²	Ireland	118	DSM-IV	HHRR	+	RR = 1.2	0.006
Waldman et al ³³	United States	122	DSM-IV	TDT	+*	OR = 1.63	0.06
Cook et al ³⁴	United States	49	DSM-III-R	HHRR	+	OR = 3.17	0.01

Note: DSM = Diagnostic and Statistical Manual of Mental Disorders; TDT = transmission disequilibrium test; HHRR = haplotype-based haplotype relative risk; OR = odds ratio; ICD-10 = International Statistical Classification of Diseases and Related Health Problems, Tenth revision; RR = relative risk.
*For combined type only.

Dopamine receptor 1 (DRD1)

Xu et al¹⁴ found that *DRD1* mutant mice exhibited heightened locomotor activity and did not respond to dopamine agonists (SKF81297) and antagonists (SCH23390), indicating that a nonaltered functioning of D₁ receptors is critical for the expression of normal motor activity. A more recent study with rats¹⁵ suggests that D₁ receptors in the prefrontal cortex may be involved in modulating attentional function, but this study has yet to be replicated. Additionally, Goldman-Racik's group¹⁷ reported an association between D₁ receptors in the prefrontal cortex and deficits in working memory, an executive function that has been studied and previously found to be disturbed in ADHD children.³⁹

The only published study of *DRD1* in ADHD¹⁰ did not implicate the receptor in increasing risk for ADHD. Further studies of this and other *DRD1* polymorphisms are needed to expound the gene's involvement in ADHD.

Dopamine receptor 2 (DRD2)

Balk et al40 used homologous recombination to generate D₂-receptor-deficient mice. These mice displayed reduced locomotor activity, as well as reduced spontaneous movements, analogous to symptoms of Parkinson's disease. Four polymorphic markers have been identified within a 25-kb haplotype system in humans.41 These markers include 3 TaqI restriction site (TaqI sites "A," "B" and "D") and 1 short tandem repeat polymorphism. In 1996, Comings et al42 reported an association between the A1 allele of the dopamine D₂ receptor gene (DRD2) and ADHD as a Tourette's syndrome associated comorbid behaviour. In addition, a relation was found between the severity and accuracy of ADHD diagnosis in subjects with Tourette's syndrome and genetic loading for specific alleles of DRD2, SLC6A3 and DBH genes (in order of relative importance based on correlation $[r^2]$ analysis). In contrast, Rowe et al43 found that higher counts of ADHD symptoms (based on Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, criteria) were associated with decreasing frequencies of the *DRD2*A1* allele. Moreover, a positive correlation was found between the A2 allele and hyperactive-impulsive symptoms, and less so for the inattentive subtype. However, when parental genotypes were used as controls for population heterogeneity, no significant results were found in Rowe's study. Rowe argues this discrepancy from

Comings' results may be the effect of multiple haplotypes with either the *A1* or *A2* alleles in linkage disequilibrium with a functional polymorphism. Other possible explanations include the heterogeneity between the 2 samples and the possibility that these results are false-negative findings. Furthermore, association of ADHD and other behavioural phenotypes with *DRD2* genotypes may depend to a significant degree on environmental exposures such as history of family stress. 44,45 Firm conclusions cannot be reached because of the small samples in both studies; larger samples with ethnically matched unrelated or family member controls are needed to validate (or refute) the authors' findings.

Dopamine receptor 3 (DRD3)

Genetic studies using animal models have shown that the dopamine D₃ receptor gene (*DRD3*) may be involved in regulating locomotor behaviour. Accili et al¹⁶ bred mice lacking functional D₃ receptors using targeted mutagenesis. They reported that *DRD3*-/- mice showed increased locomotor activity compared with heterozygotes. Ekman's et al⁴⁶ also observed such a relation in rats using a modified antisense oligodeoxynucleotide targeted against rat *DRD3* mRNA. In both studies, however, the relevance of this increased locomotor behaviour to ADHD was not extensively explored.

The *DRD3* gene has been localized in humans by Le Coniat et al⁴⁷ to chromosome 3q13.3. A single basepair polymorphism within the coding region results in an amino acid substitution (Ser \rightarrow Gly) at position 9 of the gene's amino terminal.⁴⁸ Using a hamster model, Lundstrom and Turpin⁴⁹ found that the serine allele has a significantly attenuated affinity for dopamine compared with the glycine allele. This led researchers to examine possible associations between this polymorphism and disorders implicating dopaminergic dysfunction, particularly schizophrenia.⁵⁰

Barr et al⁵¹ conducted a linkage study of 2 polymorphisms of the *DRD3* gene and ADHD: the first (mentioned earlier) alters the recognition site for an endonuclease (*MscI*) and the other, a polymorphism at intron 5, alters an *MspI* restriction site. This preliminary study does not, in fact, support any linkage between the Ser9Gly polymorphism on the *DRD3* gene and ADHD using the TDT. Similarly, more recent studies of cohorts of 150 ADHD children by Payton et al⁵² and 39 children

by Muglia et al⁵³ using TDT analysis did not identify an association or linkage. Nonetheless, future studies with larger samples are needed to reveal any link between *DRD3* and ADHD.

Dopamine receptor 4 (DRD4)

Most molecular genetic studies of *DRD4* and ADHD have focused on a VNTR polymorphism, consisting of a 48-bp repeat unit coding for an amino-acid sequence located in the third cytoplasmic loop of the receptor,⁵⁴ thought to be involved in G-protein coupling. Roughly 10 *DRD4* VNTR alleles have been identified in the global human population,⁵⁵ the most prevalent being the 4-, 7- and 2-repeat alleles, with global mean allele frequencies of 64.3%, 20.6% and 8.2%, respectively. The 4- and 7-repeat alleles, in particular, show considerable variability across populations, ranging from 0.16 to 0.96 and

0.01 to 0.78, respectively.⁵⁵ Of particular interest is the 7-repeat allele, given the low frequency of this allele and the similarly low prevalence of ADHD in Asian populations.⁵⁶ Mice lacking the *DRD4* gene have been demonstrated to be supersensitive to ethanol, cocaine and methamphetamine; in these mice, synthesis and clearance of dopamine were elevated in the dorsal striatum.⁵⁷ Van Tol et al⁴⁴ studied cloned receptor variants of *DRD4* and found different properties between the long (7 repeats) and short (2 and 4 repeats) forms of the receptor with respect to clozapine and spiperone binding. This has prompted researchers to conduct genetic association studies investigating this polymorphism and disorders in which dopamine neurotransmission may be involved.

A considerable number of studies, including both case–control and family-based association studies, have focused on the 7-repeat *DRD4* polymorphism and ADHD (Table 2). An additional 120-bp duplication

		No. of		Test of			
Study	Location	probands	Diagnostic system	association	Linkage	Statistic	p value*
Case-control asse	ociation studies						
Mill et al ⁵⁸	United Kingdom	132	DSM-IV	$\chi^{^2}$	+	OR = 6.2	0.01
Holmes et al ³⁰	United Kingdom	129	ICD-10, DSM-IV and DSM-III-R	χ²	+	OR = 1.9	0.001
Muglia et al ⁵⁹	Canada	66	DSM-IV	$\chi^{^{2}}$	+	OR = 2.5	0.01
Comings et al60	United States	52	DSM-III-R and DSM-IV	χ²	+	$\chi^2 = 6.64$	0.01
Rowe et al ⁶¹	United States	70	DSM-IV	χ²	+	$\chi^2 = 5.9$	< 0.05
Swanson et al ⁶²	United States	39	DSM-IV and ICD-10	χ²	+	$\chi^2 = 4.65$	< 0.035
Castellanos et al ⁶³	United States	41	DSM-III-R	χ²	_	$\chi^2 = 0.06$	0.81
La Hoste et al ⁶⁴	United States	39	DSM-IV	χ²	+	OR = 3.0	95% CI = 1.3-7
Family-based asso	ociation studies						
Roman et al ²⁸	Brazil	81	DSM-IV	HHRR	_	$\chi^2 = 0.37$	0.54
Payton et al ⁵²	United Kingdom	103	ICD-10, DSM-IV and DSM-III-R	TDT	_	N/A	0.75
Mill et al ⁵⁸	United Kingdom	85	DSM-IV	TDT, HHRR	_	N/A	
McCracken et al65	United States	371	DSM-IV	TDT	+	$\chi^2 = 5.4$	0.02
Barr et al ⁶⁶	Canada	82	DSM-IV	TDT	+	$\chi^2 = 15.68$	< 0.016
Holmes et al ³⁰	United Kingdom	110	ICD-10, DSM-IV and DSM-III-R	TDT	_	OR = 0.95	95% CI = 0.6-
Kotler et al ⁶⁷	Israel	49	DSM-IV	HHRR	_	LR = 7.94	0.16
Muglia et al ⁵⁹	Canada	66	DSM-IV	TDT	+	z = 1.41	0.07
Hawi et al ⁶⁸	Ireland	78	DSM-IV	HHRR	_	$\chi^2 = 0.00$	0.95
Tahir et al ⁶⁹	Turkey	104	DSM-IV	TDT	+	$\chi^2 = 2.79$	0.05
Eisenberg et al ⁷⁰	Israel	49	DSM-IV	HHRR	_	$\chi^2 = 0.14$	0.71
Faraone et al ⁷¹	United States	54	DSM-IV	TDT	+	$\chi^2 = 7.4$	0.007
Rowe et al ⁶¹	United States	70	DSM-IV	TDT	_	$\chi^2 = 0.03$	N/A
Smalley et al ⁷²	United States	129	DSM-III-R and DSM-IV	TDT	+	$\chi^2 = 4.85$	0.03
Swanson et al ⁶²	United States	52	DSM-IV	HHRR	+	$\chi^2 = 4.65$	< 0.035

polymorphism identified by Seaman et al⁷³ has also been the focus of some recent studies. 65,74 Although many of the studies identify an association between the DRD4 polymorphism and ADHD, a number of other studies do not. Faraone et al75 recently published a meta-analysis of DRD4 and ADHD. The data were derived from both family-based data (14 studies, 1665 probands) and case-control studies (8 studies, 1266 children with ADHD and 3068 controls). The odds ratio derived from the case-control studies (which indicates the odds of having the 7-repeat allele among individuals with ADHD in relation to the odds for individuals without ADHD) was 1.9 (95% confidence interval = 1.5–2.2, p < 0.001). For family-based studies, the odds ratio (an estimate of the haplotype relative risk, the odds of transmission to individuals with ADHD of the 7-repeat allele in relation to other alleles) was 1.4 (95% confidence interval = 1.1–1.6, p = 0.02). This strongly implicates *DRD4* in ADHD, highlighting the putative importance of dopamine in its etiology. The meta-analysis conducted by Faraone et al⁷⁵ indicates also that, despite the small risk conferred to individuals by the 7-repeat allele, this allele may play an important role at a population level (population attributable risk percent between 9% and 14%) because of its relatively high population frequency.

Nevertheless, *DRD4* has not been uniformly implicated in all studies of ADHD populations. For example, a recent study by Todd et al⁷⁶ examined a population-based sample of twins to establish a link between ADHD latent classes and 2 *DRD4* polymorphisms — the exon 3, 7-repeat allele and the 5′ 120-bp allele. No significant association was found between either polymorphism and the latent classes analyzed.

Molecular genetic association studies have also examined the extent to which individual ADHD traits are affected by certain genes. Novelty seeking and *DRD4* being is a common example (see Paterson et al⁷⁷ for review), but a link has not been firmly established. Of great interest is a quantitative trait study by Swanson et al⁷⁸ reporting the effects of the 7-repeat allele on specific neuropsychological behaviours believed to be trait markers of ADHD. The tasks selected were designed to probe the anterior cingulate gyrus, right dorsolateral prefrontal cortex and other areas proposed by Posner and Raichle as critical loci in the neuroanatomical network theory of attention.⁷⁹ No significant differences between those with the 7-repeat allele and those without were found, suggesting the alleles

may identify a subgroup of ADHD but not its cognitive components. However, given the small number of patients included in this study, a false-negative result cannot be ruled out.

Dopamine receptor 5 (DRD5)

Functional analysis of expressed DRD5 variants⁸⁰ has identified at least 6 amino acid substitutions, 2 of which are located in the transmembrane domains and have been associated with decreased D₅ receptor agonist binding affinity. Two research teams independently reported associations between DRD5 polymorphic loci and ADHD. Daly et al81 reported the attributable fraction for DRD5 to be 0.20 in 69 ADHD trios compared with 0.08 and 0.12 for SLC6A3 and DBH, respectively. A follow-up study by Barr et al⁸² did not reveal linkage of the 148-bp allele, but significant linkage was observed for the 136- and 146-bp alleles. Regression analysis by Comings et al¹⁰ showed that DRD5 accounted for 0.64% of the genetic variance of their ADHD population. In Payton et al's family-based study of association between various dopamine genes and ADHD,52 a trend was identified for preferential transmission of the 148bp allele, although there were no significant associations found. Conversely, Tahir et al83 reported a marginal linkage ($\chi^2 = 2.38$, p = 0.06) of the DRD5 polymorphism in their sample of children with ADHD using the TDT test. These studies suggest a possible role for DRD5 in increasing the risk for ADHD, but they remain difficult to interpret.

Catechol-O-methyltransferase (COMT)

The *COMT* gene has been of recent interest in ADHD given that the COMT enzyme is involved in the metabolic degradation of dopamine, norepinephrine and epinephrine — neurotransmitters proposed to be involved in the etiology of ADHD. Gogos et al⁸⁴ studied mice bred with a genetically disrupted *COMT* gene; *COMT*^{-/-} female mice displayed impairment in certain measures of anxiety, whereas male mutants were more aggressive, suggesting a role for *COMT* in areas of emotional and social behaviour in mice.

In humans, *COMT* has been localized to the chromosomal region 22q11.1-q11.2.85-88 Lachman et al⁸⁹ have identified a *COMT* single nucleotide polymorphism variant that causes a Val \rightarrow Met substitution at amino acid 158 of the membrane-bound form of the enzyme.

Homozygosity for methionine leads to a 3- to 4-fold reduction in *COMT* activity, compared with homozygosity for valine. The *COMT* polymorphism also creates a *Nla*III polymorphism made of 2 alleles designated *COMT*H* ("high") and *COMT*L* ("low" enzyme activity) and encoding for valine and methionine, respectively. Palmatier et al⁵⁰ studied the distribution of this polymorphism in various populations and found that the *COMT*L* allele frequency varied significantly across populations, from 0.01 to 0.62.

ADHD symptoms have been observed in children with velo-cardio-facial syndrome (VCFS),91 a condition associated with hemizygous deletions of the COMT gene region. This has spurred interest in possible associations between the COMT polymorphic locus and ADHD. In addition, it has been reported that NlaIII polymorphism may modulate neurocognitive functions, 92,93 including working memory, which is subserved by the prefrontal cortex, a region believed to be one of the brain loci disturbed in ADHD. Table 3 summarizes studies published thus far on the association between COMT and ADHD. Eisenburg et al⁹⁷ observed an association using the HHRR test between the Val high enzyme activity COMT allele and the impulsivehyperactive type of ADHD. This finding is consistent with the use of monoamine oxidase inhibitors in the treatment in children with ADHD99 — high COMT enzyme activity would make less dopamine and norepinephrine available at the synapse, whereas monoamine oxidase inhibitors increase synaptic availability of these neurotransmitters. Barr et al98 also independently studied the same COMT polymorphism in a larger sample of ADHD probands but found no association between COMT and ADHD using the TDT. Several other studies have% also refuted any association between the COMT polymorphism and ADHD. 52,94,95

Dopamine beta-hydroxylase (DBH)

Dopamine beta-hydroxylase (*DBH*) is responsible for conversion of dopamine to norepinephrine and is released along with catecholamines from the adrenal medulla and from sympathetic nerve endings. The *DBH* gene is located at chromosome 9q34¹⁰⁰ and has been closely linked to the ABO blood group. ^{101,102}

DBH polymorphisms have been studied in ADHD populations by Daly et al⁸¹ and Comings et al.^{10,42} Daly's group found that the TaqI DBH*A2 allele in the fifth intron was preferentially transmitted to ADHD children 124 times and not transmitted 95 times in 86 trios and 19 parent–proband pairs (p < 0.05). In addition, transmission of the allele was stronger among families with at least 1 parent who was retrospectively diagnosed with ADHD (relative risk = 1.49 in familial cases v. 1.20for nonfamilial cases); however, this difference was not statistically significant. Comings et al⁴² have twice investigated the effect of DBH in ADHD. In the first study, 42 the prevalence of the *DBH*B1* allele was 73.1% (p = 0.19), compared with 60.8% in non-Hispanic Caucasian controls. In the second study,10 using a multivariate linear regression analysis, DBH accounted for 0.56% of the total genetic variance of ADHD, but this was not significant (p = 0.164). In addition, a recent family-based study⁵² of 104 children with ADHD did not demonstrate an association between DBH and ADHD. The latest study, carried out by Roman et al,103 demonstrated a significant association between the TagI allele and DBH in their sample of 88 trios (HHRR test, $\chi^2 = 3.61$, p = 0.03).

Although these findings do shed interest on the possible association between *DBH* and ADHD, replication with larger samples is needed to support the association in any working model of ADHD pathophysiology.

Study	Location	No. of probands	Diagnostic system	Test of association	Linkage	Statistic	p value
Payton et al ⁵²	United Kingdom	98	ICD-10, DSM-IV and DSM-III-R	TDT	_	_	_
Manor et al94	Israel	70	DSM-IV	HHRR	_	LR = 1.74	0.19
Tahir et al ⁹⁵	Turkey	72	DSM-IV	TDT	_	$\chi^2 = 0.93$	NS
				HHRR		$\chi^2 = 2.2$	NS
Hawi et al ⁹⁶	Ireland	94	DSM-IV	HHRR	_	$\chi^2 = 0.18$	0.67
Eisenberg et al ⁹⁷	Israel	48	DSM-IV	HHRR	+	$\chi^2 = 4.72$	0.03
Barr et al ⁹⁸	Canada	77	DSM-IV	TDT	_	$\chi^2 = 1.25$	0.26

Discussion

A number of theories have postulated the involvement of brain dopamine pathways in the attention and executive functions that are believed to be altered in ADHD. Posner and Raichle's79 theory of attention involves a neuroanatomical network with a number of areas rich in dopamine innervation, including the prefrontal cortex, cingulate gyrus and anterior basal ganglia. MRI¹⁰⁴ and other imaging techniques¹⁰⁵ have identified abnormalities in these areas in children with ADHD, adding some experimental basis to this theoretical framework implicating dopamine in attention control. The most compelling evidence of the involvement of dopamine in ADHD derives from the fact that dopamine enhancers such as amphetamine and methylphenidate improve behavioural symptoms of ADHD. However, despite these converging lines of evidence implicating brain dopamine circuitry in ADHD, direct and firm evidence of its involvement remains elusive. Remarkably, this difficult and vexing problem is starting to be resolved by genetic studies. Indeed, consistent results from molecular genetic studies are pointing strongly to the possible link between 2 specific genes, SLC6A3 and DRD4, and ADHD.

The SLC6A3 VNTR polymorphism is located in the 3' untranslated region of this gene; hence, it does not affect any structural or functional aspects of the transporter protein. However, Comings¹⁰⁶ has argued on the basis of molecular genetic research of polymorphisms of other genes,107 that the different sizes of polymorphic alleles may nonetheless contribute to the regulation of gene expression. Consistent with this hypothesis, it has been reported that carriers of 2 copies of the 10-repeat allele of the SLC6A3 gene have a lower availability of the transporter.108 However, other studies have reported the opposite.¹⁰⁹ These discrepancies may be explained by differences in the demographic and clinical characteristics of the study samples and warrant further investigation to resolve them. Of particular interest, developmental differences in the level of expression of carriers of different alleles of the dopamine transporter requires further study. Evidence for a more general effect of the SLC6A3 VNTR polymorphism on transcriptional activity has been reported.100 Thus, how and when this polymorphism is involved in the modulation of the expression of the dopamine transporter or other neural pathways, including the mesocorticolimbic and nigrostriatal pathways, remains a critical question. Alternatively, this polymorphism may be completely silent but is in linkage disequilibrium with an unknown functional polymorphism. These 2 hypotheses need to be further explored by identifying other polymorphisms and testing them to identify their specific effect(s) on dopamine neurotransmission.

The DRD4 7-repeat allele has been linked to ADHD in many studies, but there have also been more recent studies refuting such an association. Given that most studies were subject to meta-analyses and the association between the polymorphism and ADHD remained robust, it is very likely that the association between DRD4 and ADHD is real. In several cases, nonreplication may be due to sample sizes that are insufficient to rule out the involvement of DRD4 or to heterogeneity in clinical characteristics of the patients studied. It is unclear whether the DRD4 VNTR polymorphism has any effect on the structure or the function of the receptor. Indeed, Asghari et al¹¹¹ found that the sensitivity to dopamine of the 7-repeat allele form of the receptor was half that of the 2- and 4-repeat variants. However, several others report no significant impact of the VNTR variants on the function of the DRD4 receptor. 112-114 Nonetheless, given that DRD4 concentrations are high in key neuroanatomical areas implicated in ADHD, and given that the VNTR polymorphism or other polymorphisms in its vicinity could conceivably contribute to the "dopamine deficit" theories of the disorder, it is likely that the gene has a significant role in perpetrating its symptoms.

Genes discussed in this paper have been implicated in other disorders involving dopaminergic dysfunction. Family studies (e.g., Biederman et al¹¹⁵) have demonstrated a high comorbidity of ADHD and Tourette's syndrome, as well as conduct, oppositional defiant, mood, anxiety and other psychiatric disorders. Most likely, these disorders, including ADHD, involve subtle anomalies within similar circuits. It is therefore possible that the observed association between ADHD and either of the 2 genes is driven by the presence of these comorbid disorders. Studies correlating the variation in phenotypic expression, both for the comorbid symptoms as well as for other aspects of the clinical variability of ADHD (therapeutic response to psychostimulant drugs, hyperlocomotion, impulsivity and inattention considered as dimensions), will be very important in the future and may lead to a better nosological dissection of this complex disorder.

There is no question that ADHD is a polygenic disor-

der. No single gene has been found to account for more than 5% of the phenotypic variance of ADHD. Furthermore, heritability studies have attributed roughly 80% of the symptoms of ADHD to genetic factors, implying that the disorder is unlikely to be caused by a single gene. Finally, and most importantly, ADHD manifests as a wide spectrum of oftentimes varying symptoms, and it is doubtful that any one gene accounts for them all. Therefore, it is plausible that allelic variants in several different genes should characterize the disorder. At least 20 genes of small effect have been studied so far.10 Compelling evidence implicates 2 of these genes, but more work is required to further confirm the role of other genes that may have less of an effect than SLC6A3 and DRD4. Increasing sample sizes, using family-based association studies and controlling for the effect of the 2 major contributors identified up to now (DRD4 and SLC6A3) may greatly help in clarifying the role of these other genes or in identifying new genetic risk factors that have not been previously studied.

ADHD is one of the rare psychiatric conditions where specific environmental factors have been implicated with relative confidence. 116-118 Assessing these risk factors in patients in genetic studies and controlling the effect of these risk factors while analyzing the genetics may help to better define the role of genes in ADHD and, possibly, to identify the mechanisms of interaction between genetic and environmental factors.

In conclusion, molecular genetic studies of ADHD are faced with the problem of heterogeneity that defines the disorder on a number of levels.119 A careful phenotyping of children with ADHD on several dimensions, including symptom and neuropsychological profiles, comorbid conditions and therapeutic response to psychostimulant drugs, will be essential to guarantee advances in the genetic and, possibly, nosological dissection of this disorder. Future studies of the genetics of ADHD must also be sensitive to the polygenic nature of the disorder. Studies of additive gene effects, for example, may provide greater insight into the effects of individual genes. To date, roughly 20 genes have been analyzed in any single ADHD population, and it is likely for the reasons described above that there are many more loci involved. Gaining a greater insight into the true genetic makeup of ADHD will require much larger samples than are currently being studied, careful selection of the control population as well as a more accurate conception of the disorder.

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References

- Brown RT, Freeman WS, Perrin JM, Stein MT, Amler RW, Feldman HM, et al. Prevalence and assessment of attentiondeficit/hyperactivity disorder in primary care settings. *Pediatrics* 2001;107:E43.
- Scahill L, Schwab-Stone M. Epidemiology of ADHD in schoolage children. Child Adolesc Psychiatr Clin N Am 2000;9:541-55.
- Swanson JM, Sergeant JA, Taylor E, Sonuga-Barke EJS, Jensen PS, Cantwell DP. Attention-deficit hyperactivity disorder and hyperkinetic disorder. *Lancet* 1998;351:429-33.
- Wolraich MI, Hannah JN, Baumgaertel A, Feurer ID. Examination of DSM-IV criteria for attention deficit/hyperactivity disorder in a country-wide sample. J Dev Behav Pediatr 1998;19:162-8.
- Brown TE. Attention-deficit disorders and comorbidities in children, adolescents and adults. Washington: American Psychiatric Press; 2000.
- Hechtman LT. Families of children with attention deficit hyperactivity disorder: A review. Can J Psychiatry 1996;41:350-60.
- Faraone SV, Biederman J. Genetics of attention-deficit hyperactivity disorder. Child Adolesc Psychiatr Clin N Am 1994;3:285-302.
- 8. Faraone SV, Biederman J. Is attention deficit hyperactivity disorder familial? *Harv Rev Psychiatry* 1994;1:271-87.
- Faraone SV, Biederman J. Neurobiology of attention-deficit hyperactivity disorder. Biol Psychiatry 1998;44:951-8.
- Comings DE, Gade-Andavolu R, Gonzalez N, Wu S, Muhleman D, Blake H, et al. Comparison of the role of dopamine, serotonin, and noradrenaline genes in ADHD, ODD and conduct disorder: multivariate regression analysis of 20 genes. Clin Genet 2000;57:178-96.
- 11. Fisher SE, Francks C, McCracken JT, McGough JJ, Marlow AJ, MacPhie IL, et al. A genomewide scan for loci involved in attention-deficit/hyperactivity disorder. *Am J Hum Genet* 2002; 70:1183-96.
- Ernst M, Zametkin AJ, Matochik JA, Pascualvaca D, Jons PH, Cohen H. High midbrain [18F]DOPA accumulation in children with attention deficit hyperactivity disorder. *Am J Psychiatry* 1999;156:1209-15.
- Giros B, Jaber M, Jones SR, Wightman RM, Caron MG. Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. *Nature* 1996;379:606-12.
- Xu M, Moratalla R, Gold LH, Hiroi N, Koob GF, Graybiel AM, et al. Dopamine D1 receptor mutant mice are deficient in striatal expression of dynorphin and in dopamine-mediated behavioral responses. *Cell* 1994;79:729-42.
- Granon S, Passetti F, Thomas KL, Dalley JW, Everitt BJ, Robbins TW. Enhanced and impaired attentional performance after infusion of D1 dopaminergic receptor agents into rat prefrontal cortex. *J Neurosci* 2000;20:1208-15.
- Accili D, Fishburn CS, Drago J, Steiner H, Lachowicz JE, Park BH, et al. A targeted mutation of the D3 dopamine receptor gene is associated with hyperactivity in mice. *Proc Natl Acad Sci U S A* 1996;93:1945-9.
- Goldman-Rakic PS, Muly EC 3rd, Williams GV. D₁ receptors in prefrontal cells and circuits. *Brain Res Rev* 2000;31:295-301.
- Hess EJ, Rogan PK, Domoto M, Tinker DE, Ladda RL, Ramer JC. Absence of linkage of apparently single gene mediated ADHD with the human syntenic region of the mouse mutant coloboma. Am J Med Genet 1995;60:573-9.
- 19. Comings DE. The clinical and molecular genetics of ADHD

- and Tourette syndrome: Two related polygenic disorders. *Ann NY Acad Sci* 2001;931:50-83.
- Terwilliger JD, Weiss KM. Linkage disequilibrium mapping of complex disease: fantasy or reality? Curr Opin Biotechnol 1998; 9:578-94
- 21. Falk CT, Rubinstein P. Haplotype relative risks: an easy reliable way to construct a proper control sample for risk calculations. *Ann Hum Genet* 1987;51(Pt3):227-33.
- 22. Ewens WJ, Spielman RS. The transmission/disequilibium test: history, subdivision, and admixture. *Am J Hum Genet* 2000;57: 455-64.
- 23. Morton NE, Collins A. Tests and estimates of allelic association in complex inheritance. *Proc Natl Acad Sci U S A* 1998;95:11389-93.
- 24. Schulze TG, Muller DJ, Krauss H, Gross M, Bauer I, Fangerau-Lefevre H, et al. Caught in the trio trap? Potential selection bias inherent to association studies using parent-offspring trios. *Am J Med Genet* 2001;105:351-3.
- 25. Giros B, Mestikawy S, Godinot N, Zheng K, Han H, Yang-Feng T, et al. Cloning, pharmacological characterization, and chromosome assignment of the human dopamine transporter. *Mol Pharmacol* 1992;42:383-90.
- Vandenbergh DJ, Persico AM, Hawkins AL, Griffin CA, Li X, Jabs EW, et al. Human dopamine transpoter gene maps to chromosome 5p15.3 and displays a VNTR. *Genomics* 1992;14:1104-6.
- Barr CL, Xu C, Kroft J, Feng Y, Wigg K, Zai G, et al. Haplotype study of three polymorphisms at the dopamine transporter locus confirm linkage to attention-deficit/hyperactivity disorder. *Biol Psychiatry* 2001;49:333-9.
- Roman T, Schmitz M, Polanczyk G, Eizirik M, Rohde LA, Hutz MH. Attention-deficit hyperactivity disorder: A study of association with both the dopamine transporter gene and the dopamine D4 receptor gene. *Am J Med Genet* 2001;105:471-8.
- 29. Curran S, Mill J, Tahir E, Kent L, Richards S, Gould A, et al. Association study of a dopamine transporter polymorphism and attention deficit hyperactivity disorder in UK and Turkish samples. *Mol Psychiatry* 2001;6:425-8.
- Holmes J, Payton A, Barret JH, Hever T, Fitzpatrick H, Trumper AL, et al. A family-based and case-control association study of the dopamine D4 receptor gene and dopamine transporter gene in attention deficit hyperactivity disorder. *Mol Psychiatry* 2000;5:523-30.
- 31. Palmer CG, Bailey JN, Ramsey C, Cantwell D, Sinsheimer JS, Del'Homme M, et al. No evidence of linkage or linkage disequilibrium between DAT1 and attention deficit hyperactivity disorder in a large sample. *Psychiatr Genet* 1999;9:157-60.
- 32. Daly G, Hawi Z, Fitzgerald M, Gill M. Mapping susceptibility loci in attention deficit hyperactivity disorder: preferential transmission of parental alleles at DAT1, DBH and DRD5 to affected children. *Mol Psychiatry* 1999;4:192-6.
- 33. Waldman ID, Rowe DC, Abramowitz S, Kozel T, Mohr JH, Sherman L, et al. Association and linkage of the dopamine transporter gene and attention-deficit hyperactivity disorder in children: heterogeneity owing to diagnostic subtype and severity. *Am J Hum Genet* 1998;63:1767-76.
- Cook EH, Stein MA, Krasowski MD, Cox NJ, Olkon DM, Kieffer JE, et al. Association of attention-deficit disorder and the dopamine transporter gene. Am J Hum Genet 1995;56:993-8.
- 35. Todd RD, Jong Y-JI, Lobos EA, Reich W, Heath AC, Neuman RJ. No association of the dopamine transporter gene 3' VNTR polymorphism with ADHD subtypes in a population sample of twins. *Am J Med Genet* 2001;105:745-8.
- 36. Swanson JM, Flodman P, Kennedy J, Spence MA, Moyzis R, Schuck S, et al. Dopamine genes and ADHD. *Neurosci Biobehav Rev* 2000;24:21-5.

- 37. Martinez D, Gelernter J, Abi-Dargham A, van Dyke C, Kegeles L, Innis RB, et al. The variable number of tandem repeats polymorphism of the dopamine transporter gene is not associated with significant change in dopamine transporter phenotype in humans. *Neuropsychopharmacology* 2001;24:553-60.
- Winsberg BG, Comings DE. Association of the dopamine transporter gene (DAT1) with poor methylphenidate response. *J Am Acad Child Adolesc Psychiatry* 1999;38:1474-7.
- Karatekin C, Asarnow RF. Working memory in childhoodonset schizophrenia and attention-deficit/hyperactivity disorder. Psychiatry Res 1998;80:165-76.
- Balk JH, Picetti R, Saiardi A, Thiriet G, Dierich A, Depaulis A, et al. Parkinsonian-like locomotor impairment in mice lacking dopamine D2 receptors. *Nature* 1995;377:424-8.
- Kidd KK, Morar B, Castiglione CM, Zhao H, Pakstis AJ, Speed WC, et al. A global survey of haplotype frequencies and linkage disequilibrium at the DRD2 locus. *Hum Genet* 1998;103:211-27.
- 42. Comings DE, Wu S, Chiu C, Ring RH, Gade R, Ahn C, et al. Polygenic inheritance of Tourette syndrome, stuttering, attention deficit hyperactivity, conduct, and oppositional defiant disorder: the additive and subtractive effects of the three dopaminergic genes DRD2, DBH, and DAT1. *Am J Med Genet* 1996;67:288.
- Rowe DC, Van den Oord EJCG, Stever C, Giedinghagen LN, Gard JMC, Cleveland HH, et al. The DRD2 TaqI polymorphism and symptoms of attention deficit hyperactivity disorder. *Mol Psychiatry* 1999;4:580-6.
- Madrid GA, MacMurray J, Lee JW, Anderson BA, Comings DE. Stress as a mediating factor in the association between the DRD2 TaqI polymorphism and alcoholism. *Alcohol* 2001;23:117-22.
- Berman SM, Noble EP. The D2 dopamine receptor (DRD2) gene and family stress; interactive effects on cognitive functions in children. *Behav Genet* 1997;27:33-43.
- Ekman A, Nissbrandt H, Heilig M, Dijikstra D, Eriksson E. Central administration of dopamine D3 receptor antisense to rat: effects on locomotion, dopamine release, and [3H]spiperone binding. *Naunyn Schmiedebergs Arch Pharmacol* 1998;358:342-50.
- 47. Le Coniat M, Sokoloff P, Hillion J, Martres MP, Giros B, Pilon C, et al. Chromosomal localization of the human D3 dopamine receptor gene. *Hum Genet* 1991;87:618-20.
- 48. Rietschel M, Nothen MM, Lannfelt L, Sokoloff P, Schwartz JC, Lanczik M, et al. A serine to glycine substitution at position 9 in the extracellular N-terminal part of the dopamine D3 receptor protein: no role in the genetic predisposition to bipolar affective disorder. *Psychiatry Res* 1993;46:253-9.
- 49. Lundstrom K, Turpin MP. Proposed schizophrenia-related gene polymorphism: expression of the Ser9Gly mutant human dopamine D3 receptor with the samliki forest virus system. *Biochem Biophy Res Commun* 1996;225:1068-72.
- Crocq MA, Mant R, Asherson P, Williams J, Hode Y, Mayerova A, et al. Association between schizophrenia and homozygosity at the dopamine D3 receptor gene. *J Med Genet* 1992;29:858-60.
- Barr CL, Wigg KG, Wu J, Zai C, Bloom S, Tannock R, et al. Linkage study of two polymorphisms at the dopamine D3 receptor gene and attention-deficit hyperactivity disorder. Am J Med Genet 2000;96:114-7.
- 52. Payton A, Holmes J, Barret JH, Hever T, Fitzpatrick H, Trumper AL, et al. Examining for association between candidate gene polymorphisms in the dopamine pathway and attention-deficit hyperactivity disorder: A family-based study. *Am J Med Genet* 2001;105:464-70.
- 53. Muglia P, Jain U, Kennedy JL. A transmission disequilibrium test of the Ser9/Gly dopamine D3 receptor gene polymor-

- phism in attention-deficit hyperactivity disorder. *Behav Brain Res* 2002;130:91-5.
- 54. Van Tol HHM, Wu CM, Guan H-C, Ohara K, Bunzow JR, Civelli O, et al. Multiple dopamine D4 receptor variants in the human population. *Nature* 1992;358:149-52.
- 55. Chang F-M, Kidd JR, Livak KJ, Pakstis AJ, Kidd KK. The world-wide distribution of allele frequencies at the human dopamine D4 receptor locus. *Hum Genet* 1996;98:91-101.
- 56. Leung PWL, Luk SL, How TP, Taylor E, Mak FL, Bacon-Shone J. The diagnosis and prevalence of hyperactivity in Chinese schoolboys. *Br J Psychiatry* 1996;168:486-96.
- 57. Rubinstein M, Phillips TJ, Bunzow JR, Dziewczapolski G, Zhang G, Fang Y, et al. Mice lacking dopamine D4 receptors are supersensitive to ethanol, cocaine, and methamphetamine. *Cell* 1997;90:991-1001.
- 58. Mill J, Curran S, Kent L, Richards S, Gould A, Virdee V, et al. Attention deficit hyperactivity disorder (ADHD) and the dopamine D4 receptor gene: evidence of association but no linkage in a UK sample. *Mol Psychiatry* 2001;6:440-4.
- 59. Muglia P, Jain U, Macciardi FM, Kennedy JL. Adult attention deficit hyperactivity disorder and the dopamine D4 receptor gene. *Am J Med Genet* 2000;96:273-7.
- 60. Comings DE, Gonzalez N, Wu S, Gade R, Muhleman D, Saucier G, et al. Studies of the 48 bp repeat polymorphism of the DRD4 gene in impulsive, compulsive, addictive behaviors: Tourette syndrome, ADHD, pathological gambling, and substance abuse. *Am J Med Genet* 1999;88:358-68.
- 61. Rowe DC, Stever C, Giedinghagen LN, Gard JMC, Cleveland HH, Terris ST, et al. Dopamine DRD4 receptor polymorphism and attention deficit hyperactivity disorder. *Mol Psychiatry* 1998;3:419-26.
- 62. Swanson JM, Sunohara CA, Kennedy JL, Regino R, Fineberg E, Wigal T, et al. Association of the dopamine receptor D4 (DRD4) gene with a refined phenotype of attention deficit hyperactivity disorder (ADHD): a family-based approach. *Mol Psychiatry* 1998;3:38-41.
- 63. Castellanos FX, Lau E, Tayebi N, Lee P, Long RE, Giedd JN, et al. Lack of association between a dopamine-4 receptor polymorphism and attention-deficit/hyperactivity disorder: genetic and morphometric analysis. *Mol Psychiatry* 1998;3:431-4.
- 64. LaHoste GJ, Swanson JM, Wigal SB, Glabe C, Wigal T, King N, et al. Dopamine D4 receptor gene polymorphism is associated with attention deficit hyperactivity disorder. *Mol Psychiatry* 1996;1:121-4.
- 65. McCracken JT, Smalley SL, McGough JJ, Crawford L, Del'Homme M, Cantor RM, et al. Evidence for linkage of a tandem duplication polymorphism upstream of the dopamine D4 receptor gene (DRD4) with attention deficit hyperactivity disorder (ADHD). Mol Psychiatry 2000;5:531-6.
- 66. Barr CL, Wigg KG, Bloom S, Schachar R, Tannock R, Roberts W, et al. Further evidence from haplotype analysis for linkage of the dopamine D4 receptor gene and attention-deficit hyperactivity disorder. *Am J Med Genet* 2000;96:262-7.
- 67. Kotler M, Manor I, Sever Y, Eisenburg J, Cohen H, Ebstein RP, et al. Failure to replicate an excess of the long dopamine D4 exon III repeat polymorphism in ADHD in a family-based study. Am J Med Genet 2000;96:278-81.
- 68. Hawi Z, McCarron M, Kirley A, Daly G, Fitzgerald M, Gill M. No association of the dopamine DRD4 receptor (DRD4) gene polymorphism with attention-deficit hyperactivity disorder (ADHD) in the Irish population. Am J Med Genet 2000;96:268-72.
- Tahir E, Yazgan Y, Cirakoglu B, Ozbay F, Waldman I, Asherson PJ. Association and linkage of DRD4 and DRD5 with attention deficit hyperactivity disorder (ADHD) in a sample of

- Turkish children. Mol Psychiatry 2000;5:396-404.
- Eisenberg J, Zohar A, Mei-Tal G, Steinberg A, Tartakovsky E, Gritsenko I, et al. A haplotype relative risk study of the dopamine D4 receptor (DRD4) exon III repeat polymorphism and attention deficit hyperactivity disorder (ADHD). Am J Med Genet 2000;96:258-61.
- Faraone SV, Biederman J, Weiffenbach B, Keith T, Chu MP, Weaver A, et al. Dopamine D4 gene 7-repeat allele and attention deficit hyperactivity disorder. Am J Psychiatry 1999;156:768-70.
- Smalley SL, Bailey JN, Palmer CG, Cantwell DP, McGough JJ, Del'Homme MA, et al. Evidence that the dopamine D4 receptor is a susceptibility gene in attention deficit hyperactivity disorder. Mol Psychiatry 1998;3:427-30.
- Seaman MI, Fisher JB, Chang F-M, Kidd KK. Tandem duplication polymorphism upstream of the dopamine D4 receptor gene (DRD4). Am J Med Genet 1999;88:705-9.
- Barr CL, Feng Y, Wigg KG, Schachar R, Tannock R, Roberts W, et al. 5'-untranslated region of the dopamine D4 receptor gene and attention-deficit hyperactivity disorder. *Am J Med Genet* 2001;105:84-90.
- Faraone SV, Doyle AE, Mick E, Biederman J. Meta-analysis of the association between the 7-repeat allele of the dopamine D(4) receptor gene and attention deficit hyperactivity disorder. *Am J Psychiatry* 2001;158:1052-7.
- Todd RD, Neuman RJ, Lobos EA, Jong Y-JI, Reich W, Heath AC. Lack of association of dopamine D4 receptor gene polymorphisms with ADHD subtypes in a population sample of twins. Am J Med Genet 2001;105:432-8.
- Paterson AD, Sunohara GA, Kennedy JL. Dopamine D4 receptor gene: novelty or nonsense? *Neuropsychopharmacology* 1999; 21:3-16.
- 78. Swanson J, Oosterlaan J, Murias M, Schuck S, Flodman P, Spence MA, et al. Attention deficit/hyperactivity disorder children with a 7-repeat allele of the dopamine receptor D4 gene have extreme behavior but normal performance on critical neuropsychological tests of attention. *Proc Natl Acad Sci U S A* 2000;97:4754-9.
- Posner MI, Raichle ME. Images of mind. New York: Sci Am Library; 1994.
- Cravchik A, Gejman PV. Functional analysis of the human D5 dopamine receptor missense and nonsense variants: differences in dopamine binding affinities. *Pharmacogenetics* 1999;9:199-206.
- Daly G, Hawi Z, Fitzgerald M, Gill M. Mapping susceptibility loci in attention deficit hyperactivity disorder: preferential transmission of parental alleles at DAT1, DBH and DRD5 to affected children. *Mol Psychiatry* 1999;4:192-6.
- Barr CL, Wigg KG, Feng Y, Zai C, Malone M, Roberts W, et al. Attention-deficit hyperactivity disorder and the gene for the dopamine D5 receptor. Mol Psychiatry 2000;5:548-51.
- 83. Tahir E, Yazgan Y, Cirakoglu B, Ozbay F, Waldman I, Asherson PJ. Association and linkage of DRD4 and DRD5 with attention deficit hyperactivity disorder (ADHD) in a sample of Turkish children. *Mol Psychiatry* 2000;5:396-404.
- 84. Gogos JA, Morgan M, Luine V, Santha M, Ogawa S, Pfaff D, et al. Catechol-O-methyltransferase-deficient mice exhibit sexually dimorphic changes in catecholamine levels and behavior. *Proc Natl Acad Sci U S A* 1998;95:9991-6.
- 85. Grossman MH, Emanuel BS, Budarf ML. Chromosomal mapping of the human catechol-O-methyltransferase gene to 22q11.1-q11.2. *Genomics* 1992;12:822-5.
- Lundstrom K, Tenhunen J, Tilgmann C, Karhunen T, Panula P, Ulmanen I. Cloning, expression and structure of catechol-Omethyltransferase. *Biochim Biophys Acta* 1995;1251:1-10.
- 87. Tenhunen J, Salminen M, Lundstrom K, Kiviluoto T, Savlainen

- R, Ulmanen I. Genomic organization of the human catechol-*O*-methyltransferase gene and its expression from two distant promoters. *Eur J Biochem* 1994;223:1049-59.
- Bertocci B, Garotta G, Da Prada M, Lahm HW, Zurcher G, Virgallita G, et al. Immunoaffinity, purification and partial amino acid sequence analysis of catechol-O-methyltransferase from pig liver. *Biochim Biophys Acta* 1991;1080:103-9.
- 89. Lachman HM, Morrow B, Sphrintzen R. Association of codons 108/158 catechol-O-methyltransferase gene polymorphism with the psychiatric manifestations of velo-cardio-facial syndrome. *Am J Med Genet* 1996;67:468-72.
- 90. Palmatier MA, Kang AM, Kidd KK. Global variations in the frequencies of functionally different catechol-O-methyltransferase alleles. *Biol Psychiatry* 1999;46:557-67.
- 91. Carlson C, Papolos D, Pandita RK, Faedda GL, Veit S, Goldberg R, et al. Molecular analysis of velo-cardio-facial syndome patients with psychiatric disorders. *Am J Hum Genet* 1997;60:851-9.
- Joober R, Gauthier J, Lal S, Bloom D, Lalonde P, Rouleau G, et al. Catechol-o-methyltransferase val-108/158-met gene variants associated with performance on the wisconsin card sorting test. Arch Gen Psychiatry 2002;59:662-3.
- 93. Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE, et al. Effect of COMT Val 108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proc Natl Acad Sci U S A* 2001;98:6917-22.
- 94. Manor I, Kotler M, Sever Y, Eisenberg J, Cohen H, Ebstein RP, et al. Failure to replicate an association between the catechol-O-methyltransferase polymorphism and attention deficit hyperactivity disorder in a second, independently recruited Israeli cohort. Am J Med Genet 2000;96:858-60.
- 95. Tahir E, Curran S, Yazgan Y, Ozbay F, Cirakoglu B, Asherson P. No association between low- and high-activity cate-cholamine-methyl-transferase (COMT) and attention deficit hyperactivity disorder (ADHD) in a sample of Turkish children. *Am J Med Genet* 2000;96:285-8.
- 96. Hawi Z, Millar N, Daly G, Fitzgerald M, Gill M. No association between catechol-O-methyltransferase (COMT) gene polymorphism and attention deficit hyperactivity disorder (ADHD) in an Irish sample. *Am J Med Genet* 2000;96:282-4.
- 97. Eisenberg J, Mei-Tal G, Steinberg A, Tartakovsky E, Zohar A, Gritsenko I, et al. Haplotype relative risk study of catechol-O-methytransferase and attention deficit hyperactivity disorder (ADHD): association of the high-enzyme activity allele with ADHD impulsive-hyerpactive people. Am J Med Genet 1999;88: 497-502.
- 98. Barr CL, Wigg KG, Malone M, Schachar R, Tannock R, Roberts W, et al. Linkage study of catechol-O-methytransferase and attention-deficit hyperactivity disorder. *Am J Med Genet* 1999;88: 710-3
- Zametkin A, Rapoport JL, Murphy DL, Linnoila M, Ismond D. Treatment of hyperactive children with monoamine oxidase inhibitors. I. Clinical efficacy. Arch Gen Psychiatry 1985;42:962-6.
- Craig SP, Buckle VJ, Lamoureux A, Mallet J, Craig IW. Localization of the human dopamine beta hydroxylase (DBH) gene to chromosome 9q34. Cytogenet Cell Genet 1988;48:48-50.
- 101. Wilson AF, Elston RC, Siervogel RM, Tran LD. Linkage of a gene regulating dopamine-beta-hydroxylase activity and the ABO blood group locus. *Am J Hum Genet* 1988;42:160-6.
- 102. Perry SE, Summar ML, Phillips JA 3rd, Robertson D. Linkage

- analysis of the human dopamine beta-hydroxylase gene. *Genomics* 1991;10:493-5.
- 103. Roman T, Schmitz M, Polanczyk GV, Eizirik M, Rohde LA, Hutz MH. Further evidence for the association between attention-deficit/hyperactivity disorder and the dopamine-betahydroxlyase gene. Am J Med Genet 2002;114:154-8.
- Filipek PA, Semrud-Clikeman M, Steingard RJ. Volumetric MRI analysis comparing subjects having attention-deficit hyperactivity disorder with normal controls. *Neurology* 1997;48:589-601.
- 105. Castellanos FX. Toward a pathophysiology of attention-deficit hyperactivity disorder. *Clin Pediatr* 1997;36:381-93.
- 106. Comings DE. Why different rules are required for polygenic inheritance: lessons from studies of the *DRD2* gene. *Alcohol* 1998;16:61-70.
- 107. Nakamura Y, Koyama K, Matsushima M. VNTR (variable number of tandem repeat) sequences as transcriptional, translational, or functional regulators. *J Hum Genet* 1998;43:149-52.
- 108. Jacobsen LK, Staley JK, Zoghbi SS, Seibyl JP, Kosten TR, Innis RB, et al. Prediction of dopamine transporter binding availability by genotype: a preliminary report. *Am J Psychiatry* 2000; 157:1700-3.
- Heinz A, Goldman D, Jones DW, Palmour R, Hommer D, Gorey JG, et al. Genotype influences in vivo dopamine transporter availability in human striatum. *Neuropsychopharmacol*ogy 2000;22:133-9.
- 110. Michelhaugh SK, Fiskerstrand C, Lovejoy E, Bannon MJ, Quinn JP. The dopamine transporter gene (SLC6A3) variable number of tandem repeats domain enhances transcription in dopamine neurons. *J Neurochem* 2001;79:1033-8.
- 111. Asghari V, Sanyal S, Buchwaldt S, Paterson AD, Jovanovic V, Van Tol HHM. Modulation of intracellular cyclic AMP levels by different human dopamine D4 receptor variants. *J Neurochem* 1995;65:1157-65.
- 112. Sanyal S, Van Tol HH. Dopamine D4 receptor-mediated inhibition of cyclic adenosine 3',5'-monophosphate production does not affect prolactin regulation. *Endocrinology* 1997;138:1871-8.
- 113. Asghari V, Schoots O, van Kats S, Ohara K, Jovanovic V, Guan HC, et al. Dopamine D4 receptor repeat: analysis of different native and mutant forms of the human and rat genes. *Mol Pharmacol* 1994;46:364-73.
- 114. Jovanovic V, Guan HC, Van Tol HH. Comparative pharmacological and functional analysis of the human dopamine D4.2 and D4.10 receptor variants. *Pharmacogenetics* 1999;9:561-8.
- 115. Biederman J, Newcorn J, Sprich S. Comorbidity of attention deficit hyperactivity disorder with conduct, depressive, anxiety, and other disorders. *Am J Psychiatry* 1991;148:565-77.
- Astbury J, Orgill A, Bajuk B. Relationship between two-year behaviour and neurodevelopmental outcome at five years of very low-birthweight survivors. *Dev Med Child Neuro* 1987;29:370-9.
- 117. Milberger S, Biederman J, Faraone SV, Guite J, Tsuang MT. Pregnancy, delivery and infancy complications and attention deficit hyperactivity disorder: issue of gene-environment interaction. *Biol Psychiatry* 1997;41:65-75.
- 118. Sprich-Buckminster S, Biederman J, Milberger S, Faraone SV, Lehman BK. Are perinatal complications relevant to the manifestation of ADHD? Issues of comorbidity and familiarity. *J Am Acad Child Adoles Psychiatry* 1993;32:1032-7.
- 119. Szatmari P. The epidemiology of attention-deficit hyperactivity disorder. *Child Adolesc Psychiatr Clin N Am* 1992;1:361-71.